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Invited Paper

Polyphenism, Phyletic Evolution, and the Structure of the Pierid Genome

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Abstract. Pierid polyphenism is discussed in the context of various contemporary issues in evolutionary biology. It is concluded that: 1. Geographic variation in the physiological control of polyphenisms represents adaptive "fine tuning" via classic microevolutionary processes. 2. The overall control of the epigenetic processes of polyphenism, as analyzed in hybridization studies, demonstrates a simple genetic architecture compatible with microevolutionary processes. 3. Phenotypic differentiation among species and among seasonal phenotypes within species does not support paleontological models linking speciation to morphological change and "genetic revolutions." 4. Concealed phenotypic plasticity is useful for inferring evolutionary history. 5. Genetic aberrations and purported atavisms may also be useful, but must be interpreted cautiously. 6. Phenotypic plasticity may contribute to stabilizing the genome, sheltering it from directional selection and thereby contributing to the perplexing phenomenon of long-term evolutionary stasis.

Introduction

> . . .whoever compares the discussions in this volume with those published twenty years ago on any branch of Natural History, will see how wide and rich a field for study has been opened up through the principle of Evolution; and such fields, without the light shed on them by this principle, would for long or for ever have remained barren.
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> Charles Darwin, in his "Prefatory Note" to the
> English edition of *Studies in the Theory of Descent*
> by August Weismann, 1882.

Charles Darwin died in 1882, the same year the English edition of Weismann's *Studies in the Theory of Descent* appeared. Weismann's book is very largely about Lepidoptera, and especially about the plasticity of their phenotypes. There is no record that Darwin himself experimented in this area, perhaps because so many of his illustrious contemporaries—on both sides of the Atlantic—were already doing so. Weismann attributes the first such experiments to Dorfmeister (1864). They thus have a history of over 120 years, all of it after the publication of *On the*

Origin of Species, and represent an important and recurrent theme in evolutionary biology. Butterfly experiments were central to the theoretical development of two of the most original and distinctive minds in the history of evolutionary biology, Weismann himself and Richard Goldschmidt. They were also critically important in the debate between Darwinians and neo-Lamarckians early in this century. Between the 1860s and World War II butterfly polyphenisms were frequently discussed in connection with almost all the scientific issues in biology. Since then, however, they have lain largely neglected while evolutionists had radically different things on their minds. Yet today the issues of greatest interest in evolutionary journals are ones familiar in the writings of both Weismann and Goldschmidt; indeed both men have been "rediscovered" and are cited with greater frequency today than for decades. This implies that butterfly polyphenisms are "relevant" to mainstream evolutionary biology again. I propose that they are, and in this paper I propose to discuss what messages they may hold for evolutionists in general.

How Polyphenism Has Been Studied

Polyphenism is the production of two or more phenotypes in individuals which do not differ in their genetic makeup. We may contrast it with polymorphism, in which by convention the phenotypes reflect underlying genetic (usually allelic) differences. (This is not the strict sense of "polymorphism" as used in the population-genetics literature, where it refers to allelic frequencies at a locus, but is a "looser" sense which comes closer to the original organismal point of reference.) Polyphenism is thus by definition an *epigenetic* phenomenon, that is, it concerns the processes whereby the genetic information is translated into a phenotype. But it is also a *genetic* phenomenon insofar as in some sense the program of translation is itself encoded in the genome, as it must be, since different species—even closely related ones—are not equipotent in this regard. This is exactly the focus of the growing discipline of developmental genetics, which tries to understand the nature of epigenetic processes, and which had its roots in the Lepidopteran studies of Goldschmidt and Kühn. There is considerable diversity of opinion today as concerns whether, or to what degree, epigenetics is an epiphenomenon of the genome. We shall return to this.

Historically, polyphenism in butterflies has been studied mostly in a proximate—mechanistic—organismic physiological manner. Let us recall briefly the very useful causal analysis of adaptation presented by Mayr (1961). Any adaptation may be studied at both *proximate* and ultimate levels of causality. Proximate here refers to the mechanisms (developmental, physiological, behavioral, etc.) whereby the adaptation is realized in the individual. Ultimate causality includes both the genetic basis for the proximate mechanisms and the ecological factors, which—serving as agents of natural selection—led to the fixation of the genes in question. There is no innate superiority in one approach as against the other; commonly they are reciprocally illuminating, as in the present case.

There is a fashion current in the evolutionary literature of distinguishing studies of "process" from those of "pattern" (Eldredge and Cracraft, 1980). This is a revival of a 19th-century tradition (Coleman, 1971). Developmental biology, epigenetics and the physiology of phenotypic determination by environmental factors are all matters of "process." But polyphenism can also be studied as "pattern." For example, one may deal literally with pattern—the relationships among pattern elements among alternate phenotypes may, for example, be studied using classical comparative methods (Shapiro, 1984a). Other "pattern" studies may seek to correlate phenotypes with seasonal, climatic and geographic factors; such correlative studies in turn enable us to construct and test hypotheses concerning function, thereby returning us to studies of "process," and comprehension of phenotypic norms of reaction permits us to predict things about seasonal and geographic patterns. And so on.

"Pattern" studies commonly are structured inductively. Thus, if we find the same geographic pattern of phenotypic variation recurring in different phyletic lineages in the same environments, we are led to infer common causality and to ask what it might be. Of course, we must be able to recognize different lineages when we see them. It is easy to say that the virtually congruent seasonal polyphenisms of *Araschnia levana* and *Pieris napi (sensu lato)* must be convergent because one is a Nymphalid and the other a Pierid; the likelihood that both got the system from a shared proximate ancestor is very remote. It is much more difficult to decide whether the seasonal polyphenisms of the *callidice* and *napi* species-groups of *Pieris* in the Holarctic are homologous, and whether either or both is homologous with the polyphenisms of the *Tatochila sterodice* species-group (also pierine) in the southern cone of South America. But it is theoretically possible and certainly worth trying.

The moral of this section is that understanding the evolution of polyphenism involves us in phylogenetic inference, whether we are systematists or not.

Mechanisms and "Fine Tuning"

> Temperature does not act on the physical constitution of the individual in the same manner as acid or alkali upon litmus paper. . .rather. . .climate, when it influences in a similar manner many succeeding generations, gradually produces such a change in the physical constitution of the species that this manifests itself by their colors and markings.
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> A. Weismann, *On The Seasonal Dimorphism of Butterflies* (Trans. R. Meldola, 1882)

An overview of mechanisms (proximate causation) for butterfly polyphenisms is given in Shapiro (1976a). In general, seasonal polyphenisms are under some combination of photoperiodic and temperature control in temperate latitudes. In tropical latitudes photoperiodic information is often of little value, and control is probably mostly related to temperature and humidity variations, Other factors, such as food quality and crowding, are relevant in specific cases.

Photoperiod is also of little value in some mid-latitude environments. One would predict on Darwinian grounds that temperature would predominate in phenotypic control in such environments, and this seems to be the case. For example, Hoffmann (1978) showed that Rocky Mountain *Colias philodice eriphyle* are essentially refractory to photoperiod, and depend on temperature, in the control of ventral hindwing melanization. (They are, however, sensitive to photoperiod in diapause induction.) This would be expected if melanization is thermoregulatory in function, since the daily regime of insolation (afternoon cloudiness) is essentially invariant over the entire flight season. Shapiro (1977a) found the same thing in coastal Californian *Pieris napi venosa*; in some years summer may actually be colder than spring due to advected fog associated with the cold California Current. Short-term prediction, based on temperature autocorrelation, is more valuable in such regimes than the longer-term seasonal prediction derived from photoperiodic information.

In the same vein, Shapiro (1978a) presented a schematic representation of the developmental options available to *Pieris napi (sensu lato)*. It seems likely that all of them have been realized in one or another population, though not all exist in any single one.

Shapiro (1978a, 1982) also showed that it is the *rule*, rather than the exception, for multivoltine polyphenic species to have multiple systems of phenotypic determination, with a hierarchy of precedence which can be rationalized adaptively. Thus, normally, short-day (long-night) phenotypic determination is irreversible by subsequent experience: a mild autumn does not imply winter is not coming. (On the other hand, warm conditions usually shorten the critical photoperiod for diapause induction, a form of reproductive gambling.) But long-day (short-night) regimes do not really "determine" phenotype—subsequent chilling will produce the hibernal phenotype. Implicit in this observation is the assumption that photoperiods do not lie, that winter is a long way off, but that means must be found to get through the present spell of adverse (cold) weather. It is scarcely surprising that phenotypes in Rocky Mountain *Pieris napi macdunnoughii*, sympatric with Hoffmann's *Colias*, are under a mixture of genetic and temperature control, with no photoperiodic influence. Photoperiod is more important in eastern *P. n. oleracea* (Oliver, 1970); in its range flight seasons are longer and there is more temporal variation in climate to deal with. Populations of the *napi* group from extreme northeastern California (Modoc County) are seemingly closely related to *macdunnoughii* but have quite distinct seasonal phenotypes with a probable photoperiodic component.

Such sorts of variation in the control of polyphenism strongly imply adaptive value and even adaptive "fine tuning" to environment. Such "fine tuning" is becoming well-documented in life-history studies (Denno and Dingle, 1981) and is the classic stuff of microevolution. In insects it is particularly well-studied as regards diapause (Dingle, 1978; Brown and Hodek, 1983).

Local adaptation, however, is not universal. In part this may be due to "phylogenetic inertia," a theme to which we shall return shortly. In part, however, the exceptions may be useful in making the rule more comprehensible. An outstanding case is the Western White, *Pieris occidentalis*.

This very abundant species seems to have a very low degree of local differentiation with respect to phenotypic determination, despite occurring from high plains to above timberline, with one to three or more broods per year. Indeed, the exceedingly dark "*calyce*" phenotypes found above tree-line in the Rockies, which have been suspected of being a different species, produce normal *occidentalis* when reared in appropriate conditions, and all *occidentalis* populations studied so far appear able to produce *calyce* in the lab, whether or not it occurs locally in nature (Shapiro, 1974a, 1975, 1976b). One explanation of the invariance is extensive gene flow, and this is a widely dispersing species (Shapiro, 1977b) whose "hilltopping" behavior would be expected to promote vertical genetic mixing, preventing the differentiation of a true alpine ecotype (Shields, 1967; Shapiro, 1974a). Presumably this would also be reflected in a low degree of differentiation at electrophoretic loci, an hypothesis we are currently testing.

Montane *Colias eurytheme* which co-occur with *C. p. eriphyle* retain their photoperiodic sensitivity, failing to parallel the seasonal biology of the other species. But *eurytheme* is only a temporary seasonal invader in these habitats, re-establishing itself by immigration every year—so local adaptation should not be expected, at least not in the short term. (This is similar to the situation in *Nathalis iole*, Douglas and Grula, 1978. In both cases phenotype is keyed to photoperiod but there is no diapause, and both species are highly vagile and largely fugitive.) Many common butterflies, especially in the western United States, are "weedy," and it is doubtful whether local populations last long enough to be considered meaningful units of evolution. Rather, they are expressions of a vast "metapopulation" (Gill, 1978) in which local adaptation is unlikely (cf. Shapiro, 1974b re *Plebeius acmon*). *Pieris rapae* has a population structure of this sort, as shown electrophoretically, but it shows clines in physiological adaptation (Vawter and Brussard, 1984; F. Slansky, pers. comm.; Shapiro, unpubl. data).

How Polyphenisms Evolve

> When we are able to change many specimens of the summer brood into the winter form by means of cold, this can only

> depend upon reversion to the original, or ancestral, form, which reversion appears to be most readily produced by cold—that is, by the same external influences as those to which the original form was exposed during a long period of time, and the continuance of which has preserved, in the winter generations, the color and marking of the original form down to the present time.
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> A. Weismann (*Ibid.*)

Polyphenisms, like diapause, appear to evolve in response to local conditions by the selective adjustment of proximate physiological mechanisms. In other words, what evolves is the "setting" of the threshold which changes the developmental instructions from phenotype 1 to phenotype 2. Microevolutionary fine tuning of this sort can be studied by hybridizing populations, races, subspecies, or species which differ in their thresholds, then working out the genetic control of the differences. The usual assumption made in such studies is that the character in question will be polygenic (i.e., best described not by simple single-locus Mendelian segregation but by the models of quantitative genetics). Because individual variation in response to environment is intrinsic in such systems (a fact of great interest, because it implies a genetic "risk-spreading" strategy in which selection perpetuates a certain level of "error" in response to environmental uncertainty; the validity of this hypothesis remains to be explored), their analysis can only be statistical in character. Studies of this sort have yet to be reported for butterfly phenotypes, though we have several in progress. They are handicapped by the fact that whole genomes, not just specific loci related to wing patterns, are mixed at hybridization; unknown developmental or genetic incompatibilities may be introduced, such that one cannot be sure that preimaginal mortality is random with respect to prospective phenotypes. Thus one cannot be sure inadvertent selection has not taken place, unless the broods are large enough for sophisticated treatment. Also, nearly all studies of incompatibility in butterfly hybrids, including various Pierid papers, are open to question because of the small numbers of replicates and the failure of the authors to report levels of incompatibility in control crosses *within* populations. Lorkovic (1978 and pers. comm.) is cognizant of the problem, but also of the practical problems in properly controlling such studies.

It is somewhat easier to study the genetic control of polyphenism in crosses between entities which are polyphenic and others which are not. To date this has been achieved only for the South American *Tatochila sterodice* species-group; it is exceedingly desirable that it be repeated for say, *Araschnia levana* X one of its monophenic Asiatic congeners. The *Tatochila* studies are still in progress, but have been reported in part in Shapiro (1984b, 1985). The "genetic architecture" of polyphenism is proving to be quite simple, involving one major locus and a variable, but thus far always small, number of modifiers. Parsons (1983) reviews the literature of the genetic basis of supposed quantitative traits, including developmental (epigenetic) ones, and finds "an underlying genetic architecture of a few genes of relatively large effect. . . .even if a trait has an outwardly continuous distribution." In other words, our results are surprising only when compared to the standard assumptions which, overall, are falsified by a large accumulation of data from a variety of organisms and traits. We shall return to this theme also, in the context of the tempo and mode of transspecific evolution.

The Relation Between Phenotype and Speciation

It should be evident that the large literature of the genetics of species differences in outbreeding animals (Ayala, 1980, 1982) gives no comfort to advocates of simplistic general models. Nonetheless, contemporary evolutionary biologists find themselves in an ongoing controversy pitting so-called "phyletic gradualism" against "punctuated equilibrium" as the "primary" mode of transspecific evolution. The latter position, generally attributed to Eldredge and Gould (1972), grew out of the observation by paleontologists that the fossil record seems to imply very long periods of morphological "stasis" ("equilibrium") within lineages, "punctuated" by abrupt speciation events. Paleontologists generally (for want of better) base new taxa on morphological change. (Neontologists do too, but to the extent that live animals can be procured and reared, they can test their inferences against the "biological species concept;" this is how we know, for example, that *Pieris occidentalis* and *"calyce"* are conspecific. One cannot do genetic experiments with dead animals.) There is thus an inevitable circularity in how paleontologists think about "speciation;" for them sibling species are beyond the pale of knowability. The consequence was a generalization that has led to all sorts of trouble: that "speciation" is essentially "instantaneous" and accompanied by profound morphologic change (which is interpreted as reflecting profound *genomic* change).

The problems with this view have been addressed extensively (Charlesworth, et al., 1982; Stebbins and Ayala, 1981, etc.). It is necessary, however, to point out that no one familiar with butterfly polyphenisms could accept this paleontological generalization. Seasonal phenotypes of butterflies are often much more different than are congeneric species whose reproductive isolation is nearly or quite complete. *Pieris napi napi* and *P. balcana* are nearly identical phenotypically, but reproductively isolated (Lorkovic, 1978). *P. n. nesis* and *P. n. japonica* are partially overlapping in Japan; they are phenotypically differentiated and rarely hybridize in nature, but are capable of doing so (Suzuki, et al., 1979). Each, however, resembles the corresponding seasonal form of the other more than it resembles its own alternate seasonal form (cf. plates in Eitschberger, 1983). The extremely complex group of "*napi*" entities on the Pacific Coast of North America is at least equally enigmatic, with seasonal polyphenism within taxa seemingly evolving more rapidly than phenotypic divergence among them (Shapiro, unpubl. data). A preliminary electrophoretic survey suggests that phenotype is more divergent in *P. protodice* and *P. occidentalis* than is the structural genome. There are no hard-and-fast rules here.

The simple genetic architecture of polyphenism in the Andean *Tatochila sterodice* species-group is mirrored in the genetics of wing-pattern differences among taxa, just as in the large literature of hybridization in the Papilios (Robinson, 1971). We have studied the inheritance of some two dozen color and pattern characters in a very extensive program of hybridization, including nearly all the possible crosses among five of the six taxa in the group, often carried to the F_3 or F_4. We have also studied the genetics of genitalic characters which have been alleged to be species-specific (Herrera and Field, 1959)—the third time the genetics of a genitalic character have been worked out, and the second time in a Lepidopteran (Shapiro and Porter, in prep.). Nearly all the taxonomically significant differences among the taxa are simply controlled and for the greater part unlinked. This includes the genes controlling sexual dimorphism, which varies greatly in the group, seemingly in a manner correlated with climate. Platt (1984) summarized generally similar results from his very extensive hybridization program in the genus *Limenitis* (Nymphalidae). Here, however, reproductive incompatibility is pronounced in certain crosses. The most incompatible combinations all involve *L. arthemis*, whose mimetic pattern is the most divergent in the genus. However, Platt presents a good argument for the incompatibility arising secondarily in sympatry, rather than being due to the loci involved in the pattern divergence *per se*: incompatibility is an inverse function of geographic distance. Given that the traditional model of reinforcement of reproductive isolating mechanisms is today under attack (e.g. Paterson, 1978), this is an especially interesting observation. (In *Tatochila* there are no barriers to quantitate against distance.) One hopes to see further analysis of compatibility data from Platt's crosses (Platt, et al., 1978; Platt, 1984).

There is a widespread belief that speciation itself is not an adaptive process, at least insofar as it occurs by differentiation of populations in allopatry. (Secondary reinforcement may be viewed as adaptive insofar as it "protects" coevolved gene complexes from disruption via hybridization, but it occurs in secondary sympatry.) The differentiation of seasonal phenotypes is inferred to be an adaptive process because of the convergences and parallel environmental and geographic gradients noted above. To date only one seasonal polyphenism, that of *Colias eurytheme*, has been shown to be adaptive by a demonstration of function (Watt, 1968, 1969); even here the actual fitness advantage has not been quantitated in the field, but the conclusions are inescapable. it is, of course, not formally possible to prove that polyphenism was originally selected for by thermoregulatory needs, even if it were shown that it is maintained by such selection today. Shapiro (1976a) showed in a small-scale survivorship study that the polyphenism of *Pieris occidentalis* was potentially adaptive to weather, but the mechanism was not explored. Roland (1982) found that ventral hindwing melanization in the univoltine *Colias* *nastes* and *Colias meadii* was correlated with levels of flight activity.

If the functional advantage of the vast majority of polyphenisms remains obscure, it is in part due to the complexity of polyphenic variation itself. Seasonal phenotypes consist of many characters, which have more or less freedom to vary independently of one another. Kingsolver (1985) discusses some of the constraints from an engineering standpoint. We may eventually be able to apply such reasoning to construct robust tests of "optimal adaptation" hypotheses in butterfly wing patterns. In the meantime, the overall character of seasonal variation does make sense, which is encouraging. *Pieris* and *Colias* have smaller wings relative to body size, and less produced wing apices, in cold-season broods. The same features are found in arctic and alpine pierines and coliadines. Anyone who has observed the behavior of these animals should realize the significance of these features. Vernal, arctic, and alpine animals fly low and very deliberately, within their "boundary layer," exercising a great deal of control over their own movements and avoiding if at all possible rising into the usually active air flow above the "boundary". Estival—and tropical lowland—animals usually fly higher, routinely leave their boundary layer, and often rise with thermals, more or less passively. Our experiments to date indicate that the wing shapes of both arctic-alpine and tropical species are pretty tightly canalized, so that rearing under bizarre conditions produces little modification (though it is often in the right direction). Thus one can see both the raw material on which selection can act in adapting either cold- or hot-climate butterflies to a regime of alternating hot and cold seasons, and vice versa. The mechanisms for such selection are in the literature (Waddington, 1961).

I have pointed out elsewhere (Shapiro, 1984a) that the highly derivative estival phenotype of the male *Tatochila vanvolxemii*, from the seasonal temperate mid-part of Argentina, is a reinvention of *Ascia*—whatever it might be "good for." *T. vanvolxemii* is partly sympatric with *A. monuste*, which is a migratory species in Argentina. One of the most astonishing aspects of the convergence is that the male *vanvolxemii* has two genetic forms of discal dot: one is smaller than the normal *Tatochila* spot and looks like the spot on *Ascia (Ascia)*; the other is larger than in other *Tatochila*—and looks like the condition in the other subgenus of *Ascia, Ganyra*!

To sum up: in the adaptive sense, the phenotypic differences within polyphenic species do not seem qualitatively different from those which characterize genetic species. The same selective pressures may apply, and only a few loci need be involved. There is no simple mapping of phenotype on genotypic differentiation in butterflies.

Phylogenetic Inference

> If this hypothesis is correct—if the variety *bryoniae* is really the original form preserved from the glacial period in certain regions of the earth, whilst *napi* in its winter form is the first secondary form gradually produced through a warm climate, then it would

> be impossible ever to breed the ordinary form *napi* from pupae of *bryoniae* by the action of warmth, since the form of the species now predominant must have come into existence only be a cumulative action exerted on numerous generations, and not *per saltum*. . . .Experiment. . . .confirmed (this)view.
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> A. Weismann (*ibid.*)

Inferring phylogeny from concealed variation is a technique which has been rediscovered almost as often as penicillin. It is, basically, merely an extrapolation of the normal pattern of phylogenetic inference, which has been so integral a part of biological education for 125 or more years that the repeated rediscovery of its implications is not at all surprising. The logical structure of Basile's (1969) inferences concerning the evolutionary history of morphology in liverwort gametophytes is identical to that of Shapiro's (1971) work on the patrimony of *Pieris virginiensis*. Due to the very different literatures in which these papers appeared (and the lack of "key words" in their titles), neither author was aware of the other until 1981 when Shapiro happened to see a reference to Basile's paper in a symposium on (!) vicariance biogeography. In response to an inquiry, Basile wrote (Sept. 15, 1981): "It is clear that our separate attempts to understand mechanisms underlying phylogeny have led to the same generalizations—that derived taxa have not necessarily lost morphogenetic capacities, and much can be learned about evolution by experimental procedures which 'free' latent or suppressed developmental processes." This approach is developed in detail by Shapiro (1981), with examples. With reference to Pieridae, it is combined with classical comparative methods to reconstruct pattern evolution (Shapiro, 1984a).

All such studies of necessity require assumptions. The first and foremost is that the direction of evolutionary change (the "polarity of morphoclines or transformation series" in cladistic jargon) can reasonably be inferred. Hennig's famous rules for this are nothing but an explicit statement of conventional (evolutionary, pre-cladistic) "common sense," but they are useful. It *is* reasonable, for example, to infer that it is easier to lose (suppress) a phenotypic character than to gain one, and that character states associated with specific ecological situations have a high probability of arising more than once in a lineage. In the case of pierine polyphenisms these statements, taken together, imply that the full ventral pattern is the primitive condition and the more or less immaculate estival pattern is derivative from it, and likely to have arisen more than once. This precise logic was used by Weismann in his analysis of the *napi* group over a century ago, and subsequently by Kautz (1955). Both these authors and Shapiro (1984a) agree on the direction of pattern evolution, though in 1882 Weismann was "on the fence" about innate physiological propensity as against natural selection, and Kautz was an explicit orthogeneticist. Anyone who retains any doubts about parallelism in the evolution of seasonal polyphenisms in the *napi* group should look at the summer forms of various taxa illustrated in color by Eitschberger (1983) Though Eitschberger is more of a classical morphotaxonomist than an evolutionist, his data unambiguously point to parallelism rather than direct homology in the loss of pattern in summer forms.

The problems of inferring parallelism and convergence from studies of the physiology and developmental biology of polyphenism have been discussed by Shapiro (1980, 1981). They are likely to remain until specific genes responsible for the phenomena have been identified and sequenced; this will be a long wait, given the poor state of Lepidopteran cytotechnology. In the meantime we can test hypotheses generated from such studies by looking for other, independent indicators of affinity. In our lab, phylogenetic reconstruction by cluster analysis of electrophoretic—genetic data has borne out the inference that the Holarctic pierines and the south American *Tatochila* evolved seasonal polyphenism convergently. There is also a suggestion that polyphenism probably evolved independently in *Pieris (Artogeia)* and *P. (Pontia, Pontieuchloia, Synchloe)* (Geiger and Shapiro, unpubl. data). Ordinary genetic studies of hybrids of the two polyphenic *Tatochila, mercedis* and *vanvolxemii*, have also borne out the hypothesis that they evolved polyphenism separately (Shapiro, unpubl. data). Unfortunately, not all searches for taxonomic congruence are so successful.

This discussion has dealt with characters "around the species level," that is, microevolution. The logic used by Basile and Shapiro has been used, however, to reconstruct phylogeny at much higher levels. Thus, Hampé (1959) experimentally restored the ankle articulation of *Archaeopteryx* in the chicken, and Kollar and Fisher (1980) convincingly demonstrated the presence of latent genes for tooth enamel synthesis in the same much-put-upon bird. All such studies are, however, haunted by the specter of Slijper's goat (Slijper, 1942a,b). (This is one of the most famous case histories in functional morphology. Born without forelegs, the animal adopted a bipedal gait and underwent a clearly adaptive series of skeleto-muscular changes paralleling those found strongly canalized in normally bipedal species. Could this imply latent ancestral bipedalism in goats? — The question is only ludicrous because one thinks one knows the patrimony of goats.) Nonetheless, it is striking, and probably significant, that the equatorial Andean pierines are not latently polyphenic (Shapiro, 1977c, 1978b). Nor is *Pieris sisymbrii* in the Nearctic, and it is not only the only obligately univoltine Nearctic pierine, it is also, electrophoretically, a very primitive *Pontia*, far back in the Holarctic dendrogram and very possibly antedating the origin of polyphenism in its sublineage.

The regularity with which interspecific pattern evolution (as in the *Tatochila sterodice* group) parallels intraspecific (polyphenic) evolution, permitting phylogenetic inference in both cases, with neither the assumption of homology nor of any "genetic revolution," underscores once again the dangers of simple generalizations about transspecific evolution. We will learn more by studying the genetics of epigenetic control in crosses of sister-species such as *Pontia daplidice* and *P. glauconome*, which differ in this regard. Alas, they first must be interfertile.

Atavism and Pattern Evolution

There are many claims of atavism (reversion) in the butterfly literature. Many of them are probably valid, but the probability of actually establishing their genetic basis is poor—and nearly all the aberrations end up on pins rather than in breeding cages. "Atavism" is a mixed concept, incorporating both back or reverse mutation and other sorts of genetic events which are of more evolutionary interest; most of this discussion will concern one such case.

The frequency of claims of reversion should give pause to those few systematists who continue to adhere to a rigid version of Dollo's "law"—the "irreversibility" of evolution. Character reversals appear to occur with great abandon in the Pieridae; not just the formalisms required for resolving contradictions in cladistics, but the real thing.

The aberration "*funebris*" appeared spontaneously in a cross of hybrid origin in the *napi* group (Lorković, 1971). Its basic effect is to convert the *Pieris* pattern to something resembling a *Colias* pattern above. Kautz (1955), by inference only from the *napi* group, and Shapiro (1984a), from pierines in general, postulated a primitive pattern of two dark lines parallel to the outer margin of the wings, and it is easy to visualize these as in turn derivative from a broad, solid coliadine-type border. Bowden (1983) interpreted "*funebris*" as a "paleomorph," analogous to the uncovering of a latent ancestral condition as described above. The actual pattern of melanization, however, does not fully support this idea. The discal spots, which are universally interpreted as the remains of the inner of the two lines, lie beyond the solid border and are fused to it on their outer edges, which is to say "*funebris*" fills in the area *between* the lines but does not reverse the suppression of the portions of the inner line which are normally missing. Since hybridization between coliadines and pierines is impossible, and no living pierine appears to produce a primitive coliadine pattern as wild type, the question of whether or not "*funebris*" is atavistic is unlikely to be resolved. However, some interesting and suggestive things can still be learned about it.

Bowden (*loc. cit.*), following Riedl (1978), interprets the genetics of "*funebris*" in the context of its hybrid origin. Riedl argues that hybridization can liberate concealed ancestral phenotypes by disrupting the gene-complexes which normally suppress their expression. In *Drosophila*, Thompson and Woodruff (1980) find that crosses of widely separated geographic strains show elevated mutation rates, and attribute this to disruption of gene-complexes which suppress mutation (or enhance repair) within populations. The precise genetic nature of "*funebris*" has thus far eluded analysis. If "*funebris*" were ancestral to all wild-type *napi* patterns, this implies that different suppressor systems would have evolved in different populations or, in effect, that the modern *napi* pattern is polyphyletic. This is best studied by putting "*funebris*" into a variety of *napi* populations, which we are doing.[1]

Such changes of genetic context, requiring successive backcrossing, also are useful in demonstrating the extent to which the expression of "*funebris*" is subject to control by the polyphenic-epigenetic system. Within the initial genetic context, the degree of susceptibility to such control seems quite limited (Bowden, 1983 and *in litt.*). If the Bowden-Riedl interpretation were correct, would we expect "*funebris*" to circumvent such control or to be subject to it? This depends on whether the liberation of the latent phenotype entails the total inactivation of its suppressors, because these would presumably have evolved in coadaptation with the polyphenic system. The stock in which Bowden has maintained "*funebris*" is relatively weakly polyphenic; its performance in the very strongly polyphenic Nearctic *napi* will be of great interest. Whatever it tells us about atavism, it already has helped to define the developmental fields for pierine wing patterns and to test the models of Kautz and Shapiro for their evolution.

Where Does Evolutionary Stasis Come From?

> . . .this. . .is equivalent to the statement that every species through its physical constitution, is impressed with certain fixed powers of variation, which are evidently extraordinarily numerous in the case of each species, but are not unlimited; they permit of a wide range for the action of natural selection, but they also limit its functions, since they certainly restrain the course of development. . .in this directive influence lies the precise reason why. . .from a given starting-point, the development of a particular species cannot now attain, even under the most favorable external conditions, any desired goal; and why, from this starting-point, given courses of development. . .must be restricted, just as a ball rolling down a hill is directed by a fixed obstacle in a direction determined by the position of the

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[1] Kautz reasoned as if *napi* could be treated in a vacuum or at least as if the *napi* complex were primitive in the Pierini. The dorsal pattern of the *napi* complex (which is most affected by *funebris*) is shared with the *rapae* group, the *brassicae* group, and with *krueperi*, whose relationships remain unclear. The preliminary electrophoretic evidence (H.-J. Geiger, pers. comm.) does not support any such generalization. In the Andean genus *Hypsochila* it appears that a "*rapae*-pattern" has evolved from a "*napi*-(or *callidice*-) pattern," but the direction of pattern evolution in the Holarctic pierines is much more open to question.

> latter, and depending on the direction of motion and the velocity at the moment of being diverted.
>
> A. Weismann (*ibid.*)

> Macroevolution turns out to be reducible to microevolutionary processes. Or at least, the theory of punctuated equilibria cannot serve as an argument for the decoupling of macroevolution from microevolution. Consequently, however, there is no reason to expect that the mechanisms of the origin of evolutionary novelties are within the reach of paleobiological analysis. The research program intended to analyze macroevolutionary change should focus first of all upon relationships of canalization and plasticity to various ecological regimes. . .to reconcile evolutionary ecology and developmental biology. Perhaps this can be done within the conceptual framework of the neo-Darwinian synthetic theory.
>
> Antoni Hoffman, in Grene (1983), p. 262.

Stasis is a real evolutionary phenomenon, not only at the morphological level as perceived by paleontologists, but at the molecular level too. Explaining it is a major unresolved problem for evolutionary biology. Van Valen (1982) reviewed the phenomenon and identified 11 possible mechanisms, of which six can be reduced to the three most-often-cited explanations: gene flow, stabilizing selection, and developmental constraints ("phylogenetic inertia"). (The others are too restrictive or improbable to be taken seriously as *general* explanations.) There are strong empirical and theoretical objections to both of the first two, and the third is poorly understood. We cannot hope to resolve the matter here, but some comments on the Pierid genome and the notion of "phylogenetic inertia" may be useful.

Developmental constraints were initially advocated by Eldredge and Gould (1972) as the most probable cause of stasis. Such "constraints," however, can be of various kinds; the most esthetically satisfying one, and the one apparently on Eldredge and Gould's minds, is that the timing of developmental events is so critical to successful ontogeny that anything which would tend to alter it is likely to be lethal (or if not, to produce a cascade of events leading to major phenotypic change, a macromutation). This is pure Goldschmidt; it should not be forgotten that his ideas, expressed to a wide audience in his 1938 book, came about equally from work on the developmental and physiological genetics of *Drosophila* and of Nymphalid wing patterns. The butterfly work has been largely forgotten, in part because it was published in German in pre-war Germany, in part because the cytotechnology never improved, and in part because it was tainted with Lamarckism.

The usual rejoinder of pro-selectionists to the "inertia" argument is that many characters have been selected radically by man, with rapid directional response and no sign of developmental disequilibrium resulting (cf. Charlesworth and Lande, 1982, also Falconer, 1960 and Lewontin, 1974). An alternative explanation is that some parts of the genome are readily selectable ("open") and others are not ("closed") (Carson, 1973, and various papers by A. Templeton).

This problem cannot be considered apart from the concept of developmental canalization (Waddington, 1957; Rendel, 1968). This concept, and even its name, recall Weismann's metaphor of the rolling ball on the incline. In an ecological context, developmental flexibility provides an economical alternative to genetic polymorphism as a response to a variable environment. If the environment is, moreover, *predictable*, phenotypic switching becomes the optimal response, doing away with both genetic load (except for "risk-spreading" or "bed-hedging" strategies, noted earlier) and the lag time of selective response. Epigenetic arrangements which allow for adaptive phenotypic plasticity, such as butterfly seasonal polyphenisms, would tend to stabilize substantial portions of the genome by doing away with seasonally-related selection; not only seasonally sensitive loci would be affected, though, since linkage groups could be tightened and the internal integration of the genome enhanced overall. To the extent that epigenetics is an epiphenomenon of the genome (rather than an emergent system property inherent in cells but independent of specific loci), it should be selectable. In the case of diapause and of polyphenism, it certainly appears to have been selected.

Pattern polyphenisms, as we have seen, may involve multiple and profound phenotypic changes (morphological, behavioral, and probably biochemical), which imply that blocks of genes are under common control mechanisms, perhaps in a manner akin to the "supergenes" of polymorphic mimics (Robinson, 1971) or the X-linked "sex package" of *Colias* (Grula and Taylor, 1980a,b), but probably not, as the phenotypic components are freer to vary among themselves. Whatever the physiology, the alternative to viewing these facts in an adaptive light is to fall back on Slijper's goat.

The suggestion is that neither developmental constraints nor stabilizing selection alone accounts for most stasis. Instead, what we may be seeing is directional selection acting on the genetic control of epigenesis, generating a system of plasticity which in turn generates its own stabilizing selection. This is the basic notion at the heart of H. G. Baker's "general purpose genotype" (1965). It is a rather different notion from Lerner's (1954) "genetic homeostasis," which is essentially an extrapolation of the idea of overdominance or hybrid vigor, but the two converge insofar as phenotypic plasticity is in a sense a form of permanent heterozygosis. (This is the reverse of genetic assimilation.)

Most such feedback processes will result in things more subtle than wing pattern polyphenisms, and may go unappreciated. Giesel *et al.* (1982) published a cautionary tale on life-history traits in *Drosophila*, noting that heritabilities and genetic correlations may behave differently in different environments. The remarkable paper of James, 1983, on the environmental component of morphological variation in birds is highly relevant, too. The idea that evolved phenotypic plasticity is a general enough phenomenon to warrant consideration as a major factor in stasis is *not* far-fetched. Indeed, it can be found in Wright (1931), one of the great papers of population genetics.

By being as conspicuous as they are, polyphenic butterflies—especially pierines—have reminded us that the organism is not necessarily a passive receiver of environmental buffeting on the tortuous road to extinction. By genomic reorganization it can potentially opt out of directional selection, at least for some things. Adaptation is potentially a two-way process.

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